

USER MANUAL

CytoQuest™CR

Non-invasive, Circulating Rare Cell Isolation and Retrieval System





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Safety Information

Users should always be aware of the following general safety precautions when operating this instrument. Using this instrument in a manner not specified in this manual may result in personal injury or damage to the instrument. Abnova (Taiwan) Corp. assumes no liability for the customer's failure to comply with these precautions.

Safety Notices

- 1. To prevent electric shock, users should keep hands, equipment, clothing, work areas dry at all times.
- 2. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- 3. The instrument should only be installed in the environment with good ventilation.
- The system must be installed and maintained by an Abnova (Taiwan)
 Corp. Technical Representative.
- 5. Wear appropriate personal protective equipment during operation (for example, safety goggles, gloves, or protective clothing).
- 6. Ensure that anyone who operates the instrument has received instructions in both general safety training for laboratories and specific safety training for the instrument.



- 7. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves.
- 8. Do not attempt to reuse the consumable parts and materials.



System Overview

Background

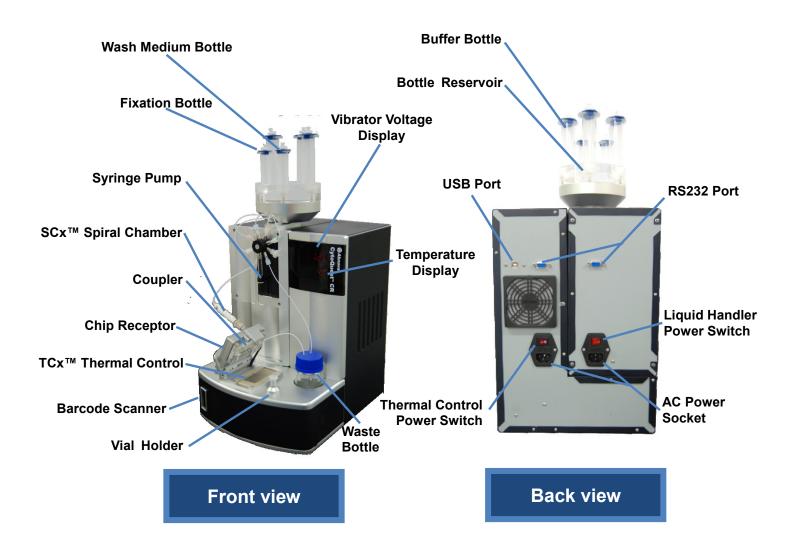
Abnova's CytoQuest™ CR is a non-invasive system for capture, enumeration, isolation and retrieval of circulating rare cells (CRCs). Three major subtypes of CRCs in translational research and clinical studies are circulating tumor cells (CTCs), circulating stem cells (CSCs), and circulating fetal cells (CFCs). A challenge for market adoption of CRCs is the efficient and reproducible identification, single cell isolation, and retrieval of highly pure and viable CRCs, with their applications supported by a wide repertoire of standardized, GMP grade bioreagents.

CytoQuest[™] CR technology utilizes SCx^{\intercal} spiral chamber, HBx^{\intercal} micromixer, antibody immobilized nanoarray, and TCx^{\intercal} thermal control to enable a multitude of CRC functions. SCx^{\intercal} spiral chamber is equipped with a non-sticky coil and a self-contained micro-vibrator for unimpeded delivery of pretreated blood sample into the nanoarray. HBx^{\intercal} micromixer provides a herringbone conduit for cell mixing. CytoChipNano is a streptavidin nanoarray which captures the CRCs for cell enumeration and single cell isolation by laser microdissection or micromanipulation. CytoChipNano CR is a specialized streptavidin nanoarray with thermo-sensitive gold coating which captures and releases the CRCs via a TCx^{\intercal} thermal control of alternating temperatures.

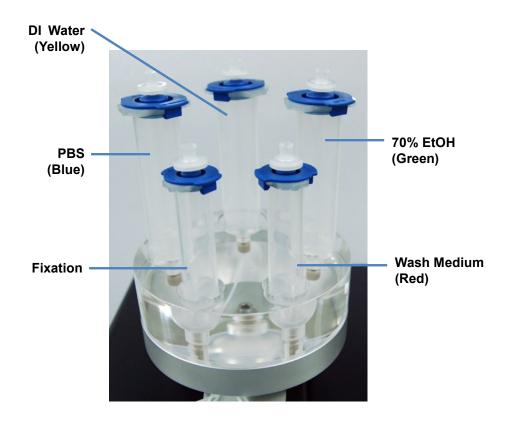
Monoclonal antibodies targeting specific surface biomarkers of circulating rare cells can be flexibly interchanged to accommodate the biomarker switch in CRC progression. This results in highly pure and viable CRCs for downstream protein characterizations, gene analyses, and cell assays. Cell enumeration is currently the only FDA approved application as a prognostic marker in breast, prostate, and colorectal cancers. In contrast, effective single cell isolation and retrieval of CRCs opens up new scope of applications in the diagnostic and pharmaceutical industry.



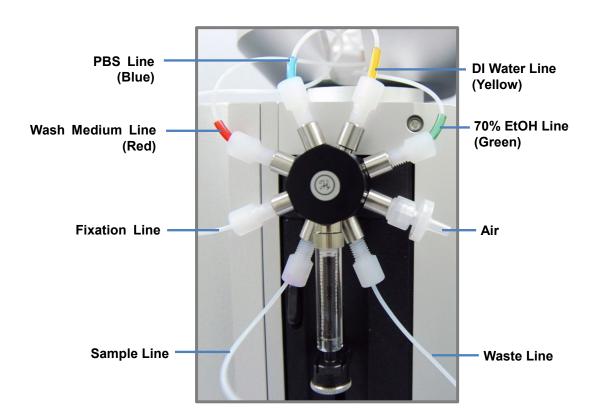
Description



• CytoQuest™ CR system, SCx™ spiral chamber, HBx™ micromixer and TCx™ thermal control are trademarks and pending patents of Abnova (Taiwan) Corporation.



Buffer Bottle



Fluidic view









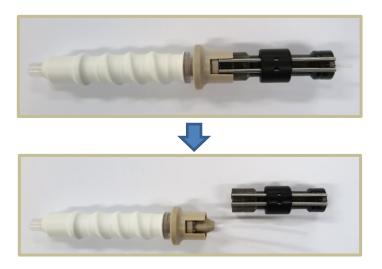




Assembling SCx[™] Spiral Chamber (top to bottom):

- Prepare Coil Holder, Micro-vibrator and Connecting Base of SCx™ Spiral Chamber.
- 2. Assemble Micro-vibrator into Coil Holder.
- 3. Assemble the part from step 2 into Connecting Base.

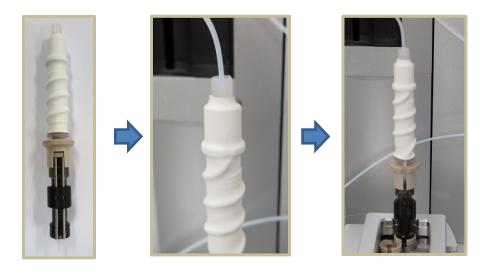




Disassembling SCx[™] Spiral Chamber (top to bottom):

 Hold the Micro-vibrator and pull off the Connecting Base.



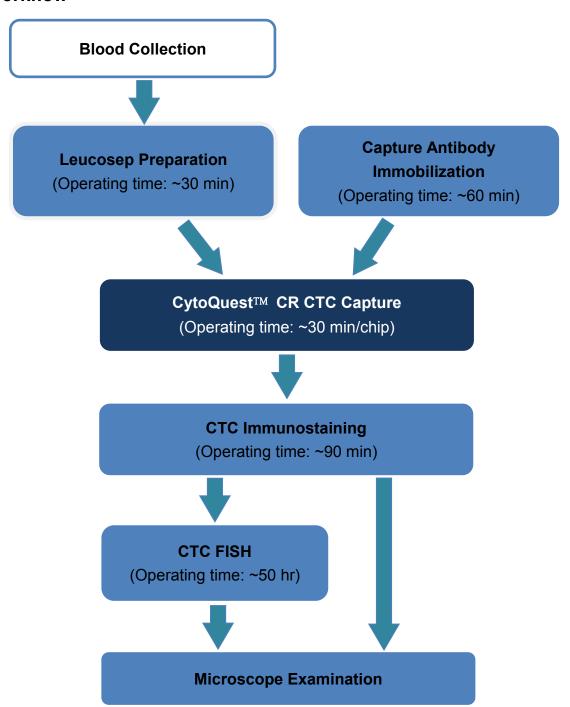


Inserting SCx™ Spiral Chamber into Chamber Port of the Coupler (left to right):

- 1. Prepare SCx™ Spiral Chamber.
- 2. Connect Sample Line to SCx™ Spiral Chamber.
- 3. Insert SCx™ Spiral Chamber into the Chamber Port of the Coupler.



Workflow



- Operate in a clean laboratory environment.
- Operate inside CytoQuest™ CR.





Required Materials

- CytoQuest™ CR (CAT# M0014-02, Abnova)
- CytoChipNano (CAT# U0095, Abnova)
- SCx[™] Spiral Chamber (CAT# U0314, Abnova)
- Coupler Plug (CAT# U0315, Abnova)
- BD Vacutainer™ Plastic Blood Collection Tubes with Sodium Heparin (CAT# 02-689-6, Fishersci)
- BD Vacutainer™ Safety-Lok™ Blood Collection Sets with Pre-Attached Holder (CAT# 02-683-21, Fishersci)
- Leucosep® (CAT# 163290P, Greiner Bio-One)
- Histopaque®-1077 (CAT# 10771, Sigma-Aldrich)
- Phosphate Buffered Saline, PBS (CAT# P-5368, Sigma-Aldrich)
- RPMI Medium (CAT# 12633-012, Invitrogen)
- Dimethyl Sulfoxide, DMSO (CAT# TS-20684, Fishersci)
- Propylene Glycol (CAT# 0215195790, Fishersci)
- 5 mL Dropper (CAT# 30006-3S1, Alpha Plus)
- Cryovial (CAT# 02-912-729, Fishersci)
- 50 mL Centrifuge Tubes (CAT# 352070, Fishersci)
- 15 mL Centrifuge Tubes (CAT# 3131-345-008, Labcon)
- Eppendorf Micro Test Tubes (CAT# 0030 121.589, Eppendorf)
- Mr. Frosty Freezing Container (CAT# 15-350-50, Fishersci)

- 2-Propanol (CAT# 109827-1L, Fishersci)
- Bovine Serum Albumin, BSA (CAT# BP9706-100, Fishersci)
- 20% Paraformaldehyde (formaldehyde) Aqueous Solution (CAT# 15715-S, Electron Microscopy Science)
- Triton X-100 (CAT# 93426-100ML, Sigma-Aldrich)
- CytoQuest™ Epithelial Cell Kit v1.0 (CAT# KA4439, Abnova):
 - ✓ Mouse Anti-EpCAM capturing antibody (Biotin)
 - ✓ Mouse Anti-PanCK detecting antibody (FITC)
 - ✓ Mouse Anti-CD45 detecting antibody (PE)
 - √ 50X Antibody Dilution Buffer (50X ADB)
- CTC Solution Kit (CAT# KA3967, Abnova):
 - √ 50X Antibody Dilution Buffer (50X ADB)
- Cartridge Pack (CAT# KA4440, Abnova):
 - ✓ CytoChipNano
 - ✓ SCx™ Spiral Chamber
- Certified Fetal Bovine Serum, FBS (CAT# 14-502F, Lonza)
- DAPI (for "CTC Immunostaining") (CAT# D1306, Invitrogen)
- Ethanol (CAT# 100983, Merck)
- Coverslip (CAT# 990, Assistent)
- IGEPAL CA-630 (CAT# I-3021, Sigma-Aldrich)
- Rubber Cement (CAT# CXA-100, KOKUYO)
- DAPI Counterstain (150ng/ml) (for "CTC FISH") (CAT# U0030, Abnova)



- Precaution
 - ✓ Be sure to read the entire manual before running the assay!



Reagent Preparation



[NOTE]

All the reagents/solutions used should be of molecular grade and sterile-filtered!

1X PBS

1X PBS consists of 8.1 mM Na₂HPO₄ • 2H₂O, 1.46 mM KH₂PO₄, 138 mM NaCl; 2.7 mM KCl, pH 7.4, at 25°C. 1X PBS without calcium and magnesium can be stored at room temperature.

WM (Wash Medium, RPMI with 5% FBS)

WM is aliquoted and kept frozen at -20°C. Once thawed, WM can be stored at 4°C for up to 3 weeks.

1X ADB

Dilute 50X ADB by adding adequate 1X PBS to 50X ADB. 1X ADB should be kept on ice or at 4°C and used within 2 days.

1X ADB/0.1% Triton X-100

Add 0.1 mL of Triton X-100 to 100 mL of 1X ADB.

4% PFA (4% Paraformaldehyde Aqueous Solution)

Dilute 20% paraformaldehyde aqueous solution with 1X PBS. 4% PFA should be aliquoted and frozen at -20°C. 4% PFA can be stored at 4°C for one week.

FM (Freezing Medium)

FM consists of 90% FBS, 5% DMSO, and 5% propylene glycol. FM should be aliquoted and frozen at -20°C. Once thawed, it can be stored at 4°C for up to 3 weeks.



2X SSC Wash Buffer

Dissolve 17.53 g of sodium chloride and 8.82 g of sodium citrate tribasic dehydrate in 700 mL deionized water. Adjust the pH to 7.0 and the volume to 1 L. Following autoclaving sterilization, 2X SSC Wash Buffer can be stored at ambient temperature.

2X SSC/0.3% NP-40

Add 3mL of IGEPAL CA-630 to 990mL of 2X SSC Wash Buffer pre-warmed to 70°C. Bring the final volume to 1 L with 2X SSC Wash Buffer. Store 2X SSC/0.3% NP-40 at ambient temperature.





Blood Collection and Handling

Phlebotomy

Access to a peripheral vein should be performed with a 23G needle. Collect 10 mL of blood sample in each Heparin tube (green cap). Gently rotate the 10 mL Heparin tube end-over-end five times immediately. Place the collected blood sample at room temperature before use.

Transportation

Blood has a maximum preservation time of 6 hours at room temperature. For example, if the blood is drawn at 9am in a hospital, it should be processed by 3pm.





6.1 Leucosep Preparation

- 6.1.1 Warm-up Histopaque®-1077 density gradient media to room temperature (RT).
- 6.1.2 Fill each Leucosep® tube with 3 mL of Histopaque®-1077 density gradient media.
- 6.1.3 Centrifuge the Leucosep® tube at 1000 x g and RT for 30 seconds. The density gradient media should be spun below the porous barrier.
- 6.1.4 Dilute the blood sample at 1:1 ratio with 1X PBS.
- 6.1.5 Carefully pour 4 mL of diluted blood sample into the prepared Leucosep® tube.
- 6.1.6 Centrifuge the Leucosep® tube at 1000 x g and RT for 10 minutes in a swinging bucket rotor.
- 6.1.7 Aspirate and discard the top layer (plasma) solution to a remnant of 5 to 10 mm in height above the PBMCs interphase.
- 6.1.8 Carefully transfer the PBMCs layer to a new 15 mL tube.
- 6.1.9 Wash the PBMCs with 10 mL of WM.
- 6.1.10 Centrifuge at 300 x g and 4°C for 10 min, then aspirate and discard the supernatant.
- 6.1.11 Repeat the washing step twice.
- 6.1.12 For CTC enumeration, re-suspend the cell pellet with 105 μL of WM and proceed to the protocol of "CTC Capture". For cell freezing and banking, please refer to "Post-Leucosep Freezing and Banking".

6.2 Post-Leucosep Freezing and Banking

- 6.2.1 Pre-cool cryogenic freezing vials at -80°C prior to cell freezing and banking.
- 6.2.2 Re-suspend the PBMCs from step 5.1.11 with 1 mL of FM pre-chilled at 4°C.
- 6.2.3 Aliquot 0.5 mL of mixed cell suspension to each cryogenic freezing vial.
- 6.2.4 Keep cryogenic freezing vials in a freezing container and place at -80°C for at least 2 hours or overnight (preferred).
- 6.2.5 Transfer cryogenic freezing vials to liquid nitrogen storage within 72 hours.





Capture Antibody Immobilization

- 7.1 Wash the CytoChipNano with 200 μ L of ethanol followed by 200 μ L of 1X PBS twice. Make sure no bubble exists in the microfluidic channel.
- 7.2 Prepare Capture Antibody Working Solution by adding 50 μL of mouse Anti-EpCAM capturing antibody (Biotin) to 50 μL of 1X PBS.
- 7.3 Pipette 100 µL of Capture Antibody Working Solution into the chip and incubate the chip at RT for 1 hour. Make sure the inlet and outlet of the chip are consistently covered with working solution throughout incubation.
- 7.4 Wash the chip with 200 µL of 1X PBS three times to remove the residual Capture Antibody Working Solution.
- 7.5 The CytoChipNano is now coated with capture antibody and is ready for use.





8.1 Parameters and Settings

- 8.1.1 Turn on the power switches of the liquid handler and thermal control of CytoQuest™ CR system.
- 8.1.2 Turn on the computer, and press the "CytoQuest CR_v2.4" icon.



8.1.3 Enter or scan in "Operator ID".

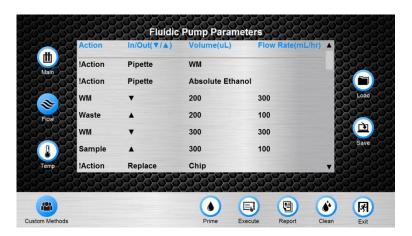




8.1.4 Press the "Custom Methods" icon.

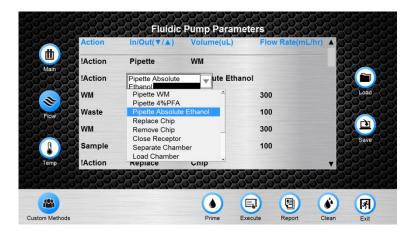


- 8.1.5 Enter or scan in "Sample ID", "CytoChipNano ID", "SCx™ Spiral Chamber ID", "Antibody Kit ID" and "Solution Kit ID".
- 8.1.6 Press the "Flow" icon on the left side of the screen.





8.1.7 Setup the Fluidic Pump Parameters.





[Note]

- For CTC Immunostaining alone, 4% PFA fixation buffer is recommended and optimized for CytoQuest™ CR.
- For CTC Immunostaining plus CTC FISH, absolute EtOH is recommended and optimized for CytoQuest™ CR.
- For other staining applications, more testing and optimization would be required.
- 8.1.8 The default temperature of the TCx[™] Thermal Control is set at 22°C. Contact an Abnova Technical Representative if you would like to alter the default setting.

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8.2 System Priming

- 8.2.1 Press the "Prime" icon on the bottom tool bar to open the "Prime" page.
- 8.2.2 Place the Clean Chip in the Chip Receptor. Insert the SCx[™] Spiral Chamber into the Chamber Port. Then Insert the "Sample line" tubing into the SCx[™] Spiral Chamber.



8.2.3 Press the "Run" icon to start the priming, and wait until this process is done.

8.3 Filling Buffer

8.3.1 Press the "Execute" icon on the bottom tool bar to open the "Execute" page.



- 8.3.2 Press the "Run" icon to start the capture process.
- 8.3.3 Follow the instruction in popup menu: "Pipette 1 mL of Wash Medium into the Wash Medium Bottle" and press the "OK" icon.





8.3.4 Follow the instruction in popup menu: "Pipette 1 mL of absolute EtOH into the Fixation Bottle" and press the "OK" icon.



- 8.3.5 The Syringe Pump withdraws 200 μL of Wash Medium. (300.0 mL/hour) from the Wash Medium Bottle.
- 8.3.6 The Syringe Pump discharges 200 µL of Wash Medium into the waste bottle via Waste Line (100.0 mL/hour).
- 8.3.7 The Syringe Pump withdraws 300 μL of Wash Medium (300.0 mL/hour) from the Wash Medium Bottle.
- 8.3.8 The Syringe Pump injects 300 µL of Wash Medium into the SCx[™] Spiral Chamber via Sample Line (100.0 mL/hour).

8.3.9 Follow the instruction in popup menu: "Open the Chip Receptor.

Remove the Clean Chip and replace with capture antibody coated CytoChipNano" and press the "OK" icon.



- 8.3.10 The Syringe Pump withdraws 10 μ L of Wash Medium (300.0 mL/hour) from the Wash Medium Bottle.
- 8.3.11 The Syringe Pump injects 10 µL of Wash Medium into the SCx[™] Spiral Chamber via Sample Line (12.0 mL/hour).
- 8.3.12 Follow the instruction in popup menu: Close the Chip Receptor." and press the "OK" icon.



8.4 Sample Loading and Capture

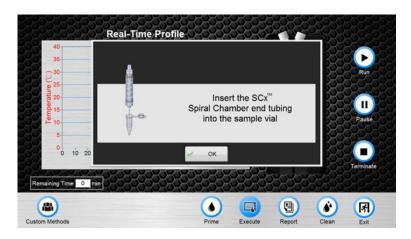
8.4.1 Follow the instruction in popup menu: "Disconnect the SCx™ Spial Chamber from the Chamber Port. Separate the Connecting Base, hold the Coil Holder, and press the "OK" icon. Do not disconnect the SCx Spiral Chamber and Sample Line.



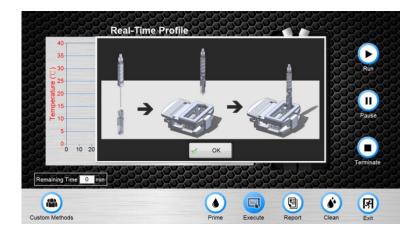
- 8.4.2 The Syringe Pump withdraws 20 µL of Wash Medium from Wash Medium Bottle (300.0 mL/hour).
- 8.4.3 The Syringe Pump injects 20 μL of Wash Medium into the SCxTM Spiral Chamber via Sample Line (12.0mL/hour).



8.4.4 Follow the instruction in popup menu: "Insert the SCx™ Spiral Chamber end tubing into the sample vial", and press the "OK" icon.



- 8.4.5 The Syringe Pump withdraws 100 μL of sample (12.0 mL/hour).
- 8.4.6 The Syringe Pump discharges 100 μL of Wash Medium into the waste bottle via Waste Line (100.0 mL/hour).
- 8.4.7 Follow the instruction in popup menu: "Assemble the SCx™ Spiral Chamber and connect to the Chamber Port" and press the "OK" icon.





- 8.4.8 The Syringe Pump withdraws 300 μL of Wash Medium from the Wash Medium Bottle (300.0 mL/hour).
- 8.4.9 The Micro-vibrator in the SCx[™] Spiral Chamber will turn on automatically.
- 8.4.10 The Syringe Pump injects 300 µL of Wash Medium into the SCx™ Spiral Chamber via Sample Line (2.4 mL/hour).
- 8.4.11 The Syringe Pump withdraws 960 µL of PBS (300.0 mL/hour).
- 8.4.12 The Syringe Pump injects 960 µL of PBS into the SCx[™] Spiral Chamber via Sample Line (4.8 mL/hour).
- 8.4.13 The Micro-vibrator in the SCx[™] Spiral Chamber will turn off automatically.

8.5 Cell Fixation

- 8.5.2 The Syringe Pump withdraws 200 μ L of absolute EtOH from the Fixation Bottle (300.0 mL/hour).
- 8.5.3 The Syringe Pump discharges 200 μL of absolute EtOH into the waste bottle via Waste Line (100.0 mL/hour).
- 8.5.4 The Syringe Pump withdraws 400 μ L of absolute EtOH from the Fixation Bottle (300.0 mL/hour).
- 8.5.5 The Syringe Pump injects 400 µL of absolute EtOH into the SCx[™] Spiral Chamber via Sample Line (4.8 mL/hour).
- 8.5.6 Follow the instruction in popup menu: "Replace the CytoChipNano with Clean Chip" and press the "OK" icon.



- 8.5.7 Allow the CytoChipNano to sit for 25 minutes to finish the fixation process.
- 8.5.8 The capture process is now finished. Please proceed to "CTC Immunostaining".



8.6 Tubing Cleaning

- 8.6.1 The Syringe Pump withdraws 500 μ L of deionized water (300.0 mL/hour).
- 8.6.2 The Syringe Pump discharges 500 µL of deionized water into the SCx[™] Spiral Chamber via Sample Line (100.0 mL/hour).
- 8.6.3 The Syringe Pump withdraws 500 μL of 70% EtOH (300.0 mL/hour).
- 8.6.4 The Syringe Pump discharges 500 µL of 70% EtOH into the SCx[™] Spiral Chamber via Sample Line (100.0 mL/hour)
- 8.6.5 The Syringe Pump withdraws 500 μL of PBS (300.0 mL/hour).
- 8.6.6 The Syringe Pump discharges 500 µL of PBS into the SCx[™] Spiral Chamber via Sample Line (100.0 mL/hour).

(28 Nov, 2014)





CTC Immunostaining

- 9.1 Pipette 200 µL of 1X PBS into the CytoChipNano three times to remove the residual fixation buffer.
- 9.2 Pipette 200 μL of 1X ADB/0.2% Triton X-100 into the CytoChipNano for 20 minutes.
- 9.3 Prepare the detecting antibody cocktail by adding 6.25 μ L of mouse Anti-PanCK detecting antibody (FITC) and 1.25 μ L of mouse Anti-CD45 detecting antibody (PE) to 250 μ L of 1X ADB.
- 9.4 Pipette 250 µL of detecting antibody cocktail into the CytoChipNano and incubate it at RT for 1 hour.
- 9.5 Pipette 200 µL of 1X PBS into the CytoChipNano three times to remove the residual detecting antibody cocktail.
- 9.6 Pipette 200 μ L of 300 nM DAPI solution into the CytoChipNano and wait for 10 minutes.
- 9.7 Pipette 200 μ L of 1X PBS into the CytoChipNano to remove the residual DPAI solution.
- 9.8 CTC Immunostaining in the CytoChipNano is now ready for "CTC FISH" or microscope observation.



10.1 Setup the Chip Incision Station with blade on the top position.



10.2 Place the CytoChipNano with its right-side up on the indent. Hold down the end of the CytoChipNano and cut the four sides of CytoChipNano with blade. Do not press the middle of CytoChipNano.



10.3 Detach the micromixer carefully from the slide.



- 10.4 Immerse the slide in 2X SSC Wash Buffer at RT for 15 minutes.
- 10.5 Immerse the slide in 2X SSC Wash Buffer at 37°C for 30 minutes.
- 10.6 Immerse the slide in 70% Ethanol at RT for 2 minutes.
- 10.7 Immerse the slide in 85% Ethanol at RT for 2 minutes.
- 10.8 Immerse the slide in 100% Ethanol at RT for 2 minutes.
- 10.9 Leave the slide air-dried.
- 10.10 Denature the FISH probe by incubating it in water bath at 75°C for 5 minutes.
- 10.11 Before applying the FISH probe, warm up the slide on the hot plate at 45°C for 5 minutes.
- 10.12 Immerse the target area with 10ul of denatured FISH probe and cover it with 22 mm x 22 mm coverslip.
- 10.13 Seal the coverslip with rubber cement.
- 10.14 Co-denature the slide on the hot plate at 80°C for 2 minutes.
- 10.15 Incubate the slide in the humidity chamber pre-warmed at 37°C for 16-48 hours.
- 10.16 Gently remove the rubber cement and coverslip.
- 10.17 Immerse the slide in 2X SSC/0.3% NP-40 at 72°C for 2 minutes.
- 10.18 Immerse the slide in 2X SSC at RT for 5 minutes.
- 10.19 Leave the slide air-dried in the dark.
- 10.20 Apply 10µL of DAPI (150ng/ml) solution to the target area and cover it with coverslip.
- 10.21 Leave the slide at -20°C for more than 30 minutes.
- 10.22 The slides are now ready for examination under fluorescence microscope.





11.1 General Maintenance

For reliable operation, keep the instrument free from dust and liquid spills. We recommend cleaning the instrument periodically. A soft cloth dampened in mild detergent is sufficient. Please operate the instrument at least once a year. If any surfaces have been contaminated with bio-hazardous material, a sterilizing solution must be used.

Buffer bottles on top of the instrument are filled with DI water (yellow bottle tubing), 70% ethanol (green bottle tubing), and 1X PBS (blue bottle tubing). The white instrument tubing is washed by these buffers. To avoid contamination, wash buffer bottles and replace the content with fresh buffers once a week. For conduct maintenance, users should always perform "System Cleaning" after operation.

11.2 System Cleaning

- 11.2.1 Press the "Clean" icon on the bottom tool bar to open the "Clean" Page.
- 11.2.2 Place the Clean Chip into the Chip Receptor.



- 11.2.3 Press the "Run" icon to start the cleaning process, and wait until it is done.
- 11.2.4 Turn off the power switches of the liquid handler and thermal control of CytoQuest™ CR system.





System Specifications

Hardware Specifications

General Specifications		
Dimensions:	475.6mm(h) x 340.8mm(d) x 271.8mm(w)	
Net Weight:	18 kg	
Power:	100-240VAC ; 85-250VAC	
Current:	1A typical (1.5A peak); 3.5A typical (4A peak)	
Operating	60°F to 05°F (45°C to 25°C)	
Temperature:	60°F to 95°F (15°C to 35°C)	
Programable Thermo	4.40°C Variable Time Span	
Control Profile:	4-40°C, Variable Time Span	

Environmental Specifications				
Storage	-5°F to 158°F (-20°C to 70°C)			
Temperature:				
Operation Altitude:	Up to 2000m			
Operation	For Indoor Lloo			
Environment:	For Indoor Use			
Operating Humidity:	20% to 90% Relative Humidity, Non-Condensing			
Storage Humidity:	20% to 95% Relative Humidity, Non-Condensing			



Software Specifications

General Specifications		
Scanner	Scanning Capability to Read QR Code/Barcode	
Function:	Drogress and Chrid Handling	
Status Display:	Progress and Fluid Handling	
System Clean:	Prime and Clean	
Report	Print and Export File	
Generation:	Till and Export no	
	LabVIEW Run-Time Engine 2011 (Standard RTE)	
Necessary Driver:	NI-VISA Run-Time Engine 5.4	
	PL2303_Prolific_DriverInstaller_v1.9.0	

Touchscreen Laptop Specifications

General Specifications			
	Windows 8/7/Vista (32-bit and 64-bit)		
Operating System:	Windows XP SP3 (32-bit)		
Operating System:	Windows Server 2003 R2 (32-bit)		
	Windows Server 2008 R2 (64-bit)		
Processor:	Pentium III/Celeron 866 MHz or Equivalent		
Memory:	256MB Minimum (512MB Recommended)		
Free Disk Space:	1GB		
Interfaces/USB Ports:	USB 3.0 Port *1		